Roth, G. (1974): Kritik der Verhaltensforschung. München: Beck.
Sachs, L. (1978): Angewandte Statistik, Statistische Methoden und ihre Anwendung. Berlin, Heidelberg, New York: Springer.
Smith, R. H. (1972): Wildness and Domestication in *Mus musculus*: A Behavioral Analysis. J. Comp. Physiol. Psych. **79**, 22–29.
— (1978): Open-Field Freezing as a Stable Parameter of Wildness in *Mus musculus*. Behav. Biol. **23**, 67–74.
Smith, R. H.; Connor, J. L. (1974): The Inheritance of Behavioral Wildness in House Mice (*Mus musculus* L.). Anim. Learn. Behav. **2**, 249–256.
— — (1978): Behavioral Effects of Laboratory Rearing in Wild *Mus musculus*. Behav. Biol. **24**, 387–392.
Stresemann, E. (1970): Exkursionsfauna von Deutschland. Wirbeltiere. Berlin: VEB Volk und Wissen.
Walsh, R. N.; Cummins, R. A. (1976): The Open-Field Test: A Critical Review. Psychol. Bull. **83**, 482–504.
White, C. (1952): The Use of Ranks in a Test of Siginificance for Comparing Two Treatments. Biometrics **8**, 33–41.
Wieser, W. (1976): Konrad Lorenz und seine Kritiker. München: Piper.

Anschrift des Verfassers: Prof. Dr. M. Walter Schäfer, Fachbereich Biologie (Zoologie) der Universität, Siesmayerstr. 70, D–6000 Frankfurt a. M.

Behavioural and neurophysiological studies of the olfactory sensitivity in the albino mouse

By Christel Schmidt

Zoological Institute, University of Bonn

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Abstract

Studied was the olfactory sensitivity of ♂♂ mice by means of training experiments and electrophysiological investigations. Three mice were investigated with both methods, and the behavioural and neural thresholds were found to be in the same range. Neural thresholds were determined in 24 animals for geraniol ($5 \times 10^7 - 5 \times 10^9$ molecules/cm^3 air), butyric acid ($7 \times 10^7 - 7 \times 10^9$ mol./cm^3) and butyric methyl ester ($4 \times 10^9 - 4 \times 10^{11}$ mol./cm^3) by studying evoked potential measurements and analysis of bulbar oscillations.

Introduction

Considering the complex significance of the olfactory sense in rodents, it is surprising that only few investigations deal with the olfactory sensitivity of this mammalian order. Behavioural experiments are performed with guinea pigs (Matthes 1932) and rats (Gruch 1957; Moulton and Eayrs 1960; Moulton 1961) to establish olfactory thresholds, whereas in albino mice neural response thresholds have been determined (Schmidt 1978; Schmidt and Schmidt 1980).

The results of these few investigations are even difficult to compare, as the threshold values were obtained with different methods. Whereas in the behavioural studies the perceptive ability of the animals is investigated, the electrophysiological experiments document a neural reaction of a certain brain area. To evaluate these results it is necessary to compare behavioural and neural threshold data. Investigations of the same individual with different methods especially allow a more accurate estimation of its olfactory ability.

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Material and methods

The experiments were carried out with ♂♂ albino mice, 6–8 months old (body-weight 30–38 g). Three mice were trained for the behavioural studies; the neural thresholds of these animals were determined after completing the training experiments. In addition, neural olfactory thresholds were established in 24 mice.

Training experiments

The transparent Y-maze for the behavioural tests (fig. 1) consisted of a starting box and two destination boxes, connected by tunnels, and a central triangle. Shock grids were placed in front of both destination boxes (stimulation frequency 20 Hz; voltage 20–50 V). The clean (charcoal filter, water) and temperature constant (22–23 °C) air stream was divided before reaching the bottles with odour and with the control substance (solvent of the odourant). The flow velocity of the air stream was 10 cm^3/s. The outlets ended 1 cm in front of the shock grids. Olfactory thresholds were established for butyric acid (BA) and geraniol (GER). The choice apparatus was cleaned between each trial with Extran^R and washed with fresh air; stale air was permanently sucked out by a funnel at the bifurcation.

The animals were trained to select the odourous air stream. Every day the mice performed 3 warm up runs and 20 test trials. The location of odour- and control stream was randomly changed. For each concentration tested (log. unit steps) 60 trials were analysed (chance level: $p < 0.001$; z-test). Olfactory adaptation was diminished by an interval of 8–10 minutes between the trials.

At high odour concentrations, the 3 mice already made their decision at the bifurcation, while at low concentrations after sniffing at the outlets. Correct choices were rewarded with food, whereas wrong ones were punished with a slight electrical shock.

Electrophysiological experiments

The electrical activity from the olfactory bulb was recorded with electrodes (tungsten wire) insulated up to the tip (∅ 50 µm; resistance 20–30 kΩ), permanently implanted in the brain and held in position by a mass of dental cement that also enclosed two small steel hooks anchored in the parietal bone. A

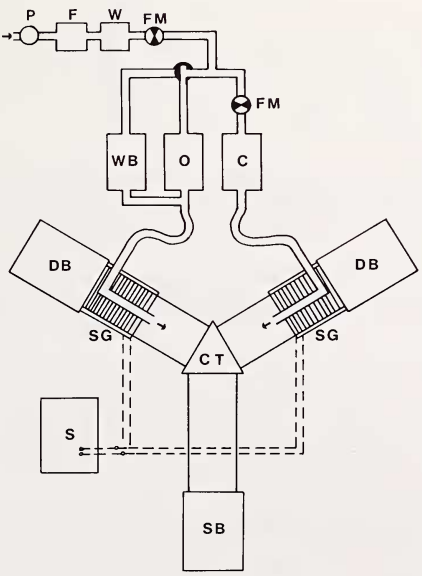

Fig. 1. Two choice training apparatus. SB = starting box; S = shock generator; CT = central triangle; SG = shock grid; DB = destination box; C = control substance; O = odourant; WB = wash bottle; FM = flow meter; W = water bottle; F = charcoal filter; P = pump

metal clamp attached to the ipsilateral ear served as the reference electrode (Schmidt 1978). Olfactory responses in the waking mice were recorded 24 hours after implantation. The olfactory stimulus (duration 1 s) was presented with a motor-driven syringe-olfactometer. Three substances were tested with the electrophysiological methods – butyric acid (BA), butyric methyl ester (BME) and geraniol (GER) – at 3 minutes intervals, beginning with subthreshold concentrations. Control stimuli (pure air) were presented between the odour stimuli at irregular intervals. A continuous flow of air from the

side, together with a funnel mounted above the animal to draw air away, ensured the odour neutrality of the surroundings. The activity of the olfactory bulb was recorded with the standard apparatus (oscilloscope, pen writer). The criterion for a positive neural response to an odour stimulus was the occurence of evoked potentials in the form of a negative deflection at the end of the inspiration.

Results

Behavioural experiments

Pilot tests had shown that the animals behave differently when confronted with diverse odour concentrations. They rejected butyric acid and geraniol in low dilutions (10^{-1} vol.%) and avoided even high dilutions of butyric methyl ester, so that this odourant was unsuitable for these experiments. The training started with geraniol (10^{-2} vol.% $\triangleq 5 \times 10^{11}$ molecules/cm^3 air). In the beginning the door of the negative destination box was closed, so that the animals were forced to correct misjudgements. Nevertheless both boxes had to be opened after some time, since the mice localized the closed box, possibly by

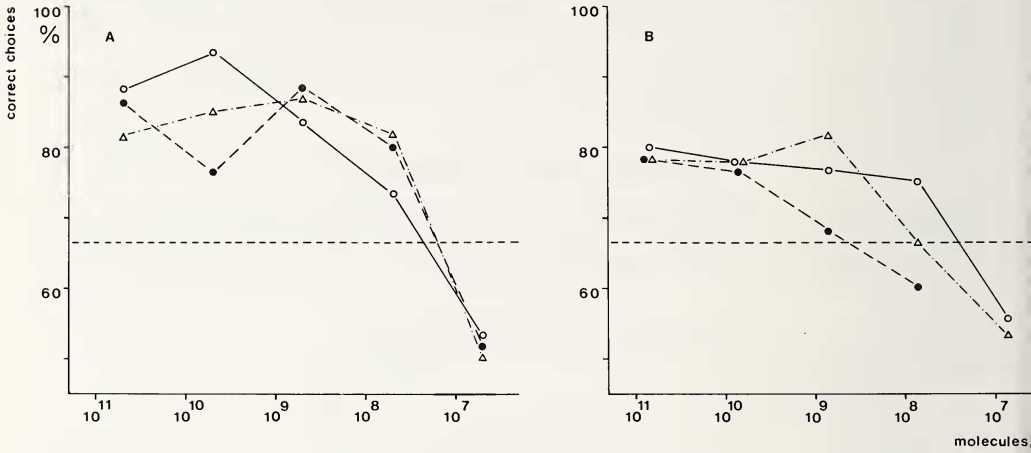

Fig. 2. Performance of 3 male albino mice trained to select geraniol (A) and butyric acid (B). Ordinate: percentage of correct choices; abscissa: concentration of the test substance (odour molecules/cm^3 air); z = chance level ($p < 0.01$; z-test). The 3 males are identified by different symbols

acustical cues. The first obvious preference for the odourous stream could be recognized after ca. 130 trials. Threshold measurements started when the animal showed more than 80 % correct choices within 60 consecutive trials. This training level was reached after 240–300 trials. In the main experiments, each concentration was tested by 60 trials.

The performance level is significantly positive ($p < 0.001$) in all animals up to a concentration of 5×10^8 molecules geraniol/cm^3 air. At the next lower concentration (5×10^7 mol./cm^3) all animals choose randomly (fig. 2a). Although the second substance (BA) was immediately accepted, the performance level remains lower than for geraniol (ca. 80 %) even after 10 days training. Individual differences between the animals are obvious with lower concentrations. While the performance with a concentration of 7×10^9 molecules BA/cm^3 is significantly positive in all mice, only 2 of them are able to detect 7×10^8 mol./cm^3; with 7×10^7 mol./cm^3 choices are at random (fig. 2b).

Neurophysiological threshold measurements

With low resistance electrodes two characteristic potentials are recorded from the olfactory bulb of the mouse: 1.) a slow fluctuation consisting of a negative-going wave during

inspiration and a positive wave during expiration, and 2.) regular oscillations beginning at the end of the inspiration phase and continuing for most of the expiration phase (phase B; SCHMIDT 1978).

Simultaneous recordings from the 2 bulbs reveal that both the background fluctuation and the oscillations result from stimulation of the nasal mucosa. When one side of the nose is reversibly blocked, both the respiration-linked and the superimposed oscillations in the ipsilateral bulb vanish, whereas in the contralateral bulb the normal resting activity persists (fig. 3).

As pilot experiments have shown, the bulbar potentials are very sensitive to anesthetics and neuroleptics. For instance 160 mg/kg body weight Evipan^{R} (sodium hexobarbital; 15–20 min anesthesia) abolish all potentials; with Megaphen^{R} (chlorpromazine) evoked potentials can only be elicited by intense odour stimuli. Therefore, all the experiments are carried out with animals unaffected by any drugs.

The evoked potential, a negative deflection occuring at the onset of the oscillations when stimulated with an odourant, can be used to determine the neural olfactory thresholds. The pattern of the bulbar potentials change, when recorded in different areas of the olfactory bulb. Recordings from deeper layers are characterized by large oscillations and relatively inconspicious evoked potentials; when the tip of the electrode is placed in the mitral-cell region, the evoked potentials are extremely large, while the oscillations are small and sometimes not detectable at all. Nevertheless, the threshold concentration, at which the evoked potential is first discernible, is independant of the location of the electrode in the bulb.

In the 3 mice investigated with both methods, for geraniol neural and behavioural thresholds are exactly the same (5×10^8 mol./cm^3 air). For butyric acid two animals showed the same thresholds (7×10^8 mol./cm^3) independant of the method used, while the neural threshold of one animal is lower by a factor of 10 (7×10^7 mol./cm^3). The 24 males

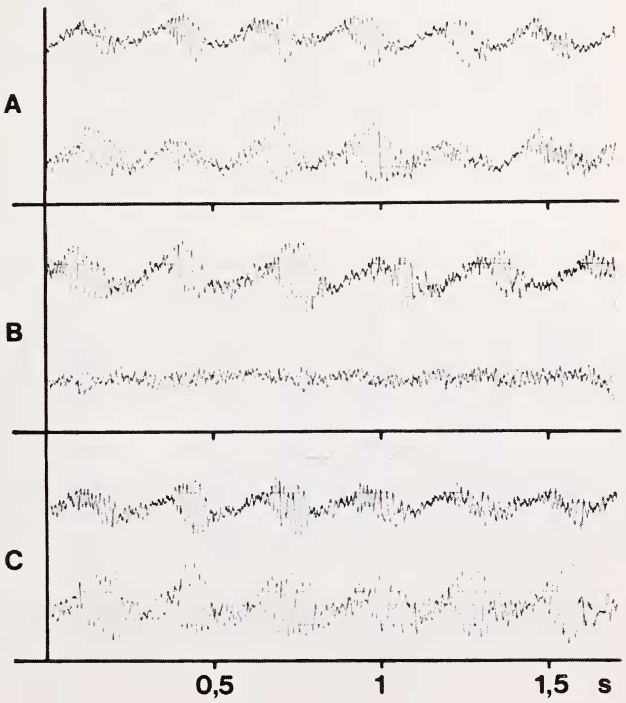

Fig. 3. Normal activity of the olfactory bulbs, recorded simultaneously on both sides. A: both nostrils open; B: one nostril closed. In the bulb ipsilateral to the closed nostril the characteristic potential fluctuations have disappeared. C: when the nostril is reopened both bulbs again exhibit the normal activity pattern

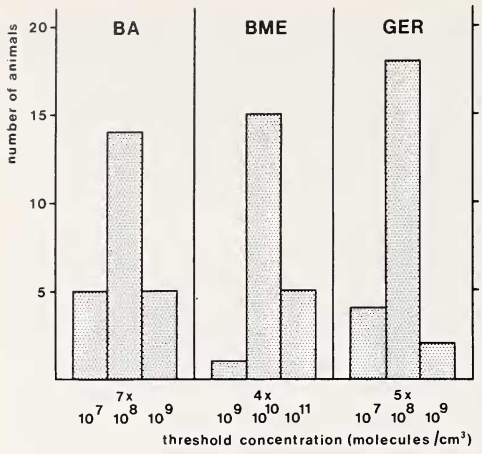

Fig. 4. Neural thresholds measured by means of evoked potentials. Ordinate: number of animals with the indicated thresholds; abscissa: threshold concentration (odour molecules/cm³ air); BA: butyric acid; BME: butyric methyl ester; GER: geraniol

used only for the electrophysiological studies, show the first reactions to BA between 7×10^7 and 7×10^9 mol./cm³; the thresholds measured for BME are between 4×10^9 and 4×10^{11} mol./cm³ and with geraniol 5×10^7 to 5×10^9 mol./cm³ air are necessary to elicit a neural response in the olfactory bulb (fig. 4).

The evoked potential is not the only change in bulbar activity to be observed during olfactory stimulation, the number and frequency of the oscillations change as well. In the presence of subthreshold odour concentrations, the number of oscillations per breath is nearly uniform before, during and after odour presentation, but with above-threshold stimuli, there is a distinct reduction in the number of oscillations during the stimulus. The maximal reduction with BME amounted to 71 % (fig. 5a) from the lowest concentration (10^{-7} vol.% $\triangleq 4 \times 10^8$ mol./cm³; 4.82 oscillations per breath) to the highest (10^{-1} vol.% $\triangleq 4 \times 10^{14}$ mol./cm³; 1,4 o/b). This change in oscillation frequency, in inverse proportion to the odour concentration, is just as striking for the other substances (BA: 7×10^6 mol./cm³; 5.22 o/b and 7×10^{13} mol./cm³; 0.97 o/b — GER: 5×10^6 mol./cm³; 4.28 o/b and 5×10^{12} mol./cm³; 1.43 o/b).

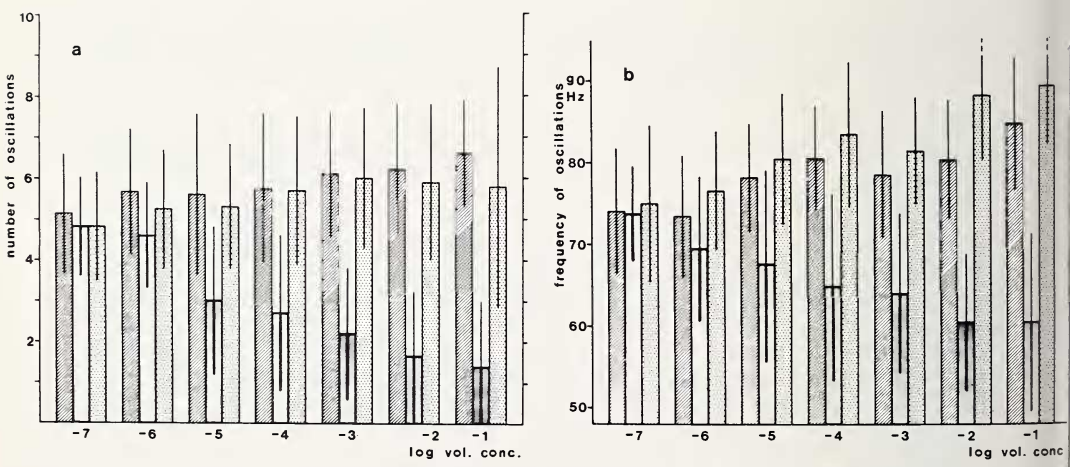

Fig. 5. a: number of oscillations/breath ($\bar{x}$) at different odour concentrations (BME); b: Mean frequency of oscillations/breath ($\bar{x}$) at different concentrations of BME. Abscissa: concentration of BME (log. vol.%); hatched: 1 s before stimulation; shaded: 1 s during stimulation; punctuated: 1 s after odour presentation; vertical lines: standard deviation

The mean oscillation frequencies calculated for the same periods of time are also closely correlated with stimulus intensity. In all 5 mice tested, an increase in odour concentration is accompanied by a reduction in frequency during stimulus presentation. In the case of BA and BME this phenomenon first appears when the concentration is raised to 7×10^8 mol./cm^3 BA resp. 4×10^9 mol./cm^3 BME ($\hat{=} 10^{-6}$ vol.%; fig. 5b); (probability of error in assuming that the frequency during the second preceding olfactory stimulation is greater than that during stimulation: BA, $p < 0.001$; BME, $p < 0.01$). With geraniol the first statistically significant frequency reduction occurs with 5×10^8 mol./cm^3 ($p < 0.001$).

The absolute frequency reduction obtained with the highest concentration presented (10^{-1} vol.%), as compared with the unstimulated or subthreshold situation, is 11 Hz for BA, 13 Hz for BME, and 15 Hz for GER. In the second following the stimulus, the oscillation frequency rises above the unstimulated level by almost the amount of the preceding fall (BA, 10 Hz; BME, 14 Hz; GER, 10 Hz). This overshooting recovery, which leads to a gradual increase in unstimulated frequency as the series of stimuli at increasing concentrations proceeds, are interpreted as effects of adaptation.

Discussion

Investigations of the efficiency of sensory organs in mammals may lead to very diverse results, when different methods are applied. Especially the differences between neurophysiological and behavioural methods may amount to great values, since the former give electrical responses of small neural areas or units and the latter consider the whole animal. Therefore, the results of such experiments are not always comparable. For instance, a behavioural threshold gives an indication of the perceptual ability, whereas a neural threshold does not mean per se that the animal can actually perceive a given threshold stimulus. Various methods – behavioural as well as electrophysiological ones – should be used for evaluating threshold values.

Training experiments or conditioning methods have mainly been used to estabish threshold values for olfaction; data on neural thresholds are only available for mice (Schmidt 1978; Schmidt and Schmidt 1980). As the potentials in the olfactory bulb are strongly affected by anesthetics, only animals which are fully awake are suitable for these studies. Therefore, permanently implanted electrodes prove to be most suitable for threshold measurements. They have the advantage of registering the bulbar activity in unanesthetized animals and allow to work with the same individual for several weeks (Schmidt und Schmidt 1982).

Two parameters were used in the present study to determine threshold values: The evoked potentials and the change of oscillation frequency. Both potentials seem to be generated in different parts of the olfactory bulb. While the negative potential, evoked by a sudden olfactory stimulus, can be recorded best in the outer layers of the Bulbus olfactorius, the amplitude of the oscillations increases in the deeper layers of the bulb. Nevertheless, the olfactory thresholds obtained with both potentials match.

Comparing the results of the behavioural and the electrophysiological studies it becomes evident that all the thresholds are in the same order of magnitude. So it seems admissible to conclude that the threshold concentrations obtained are in a reliable range.

As olfactory threshold measurements in mammals are very scarce, comparable data are only available for butyric acid. The results obtained in these studies indicate that the olfactory sensitivity of the laboratory mouse for BA is in the same range as in other small macrosmatic mammals, for instance the european hedgehog (Bretting 1972). These low threshold values emphasize the importance of olfaction in the life of the mouse.

Zusammenfassung

Verhaltensphysiologische und elektrophysiologische Untersuchungen zur Riechfähigkeit von Albinomäusen

Bei ♂♂ Albinomäusen wurde die Riechleistung mit verhaltensphysiologischen und elektrophysiologischen Methoden untersucht. Die olfaktorischen Schwellen (Geraniol, Buttersäure) der drei Tiere, bei denen anschließend an eine Geruchsdressur Ableitungen vom Bulbus olfactorius durchgeführt wurden, lagen bei beiden Methoden im gleichen Größenordnungsbereich. Bei 24 weiteren Mäusen wurden neurale Schwellen mit Hilfe evozierter Potentiale sowie durch eine Frequenzanalyse der für den Bulbus olfactorius charakteristischen Oszillationen bestimmt. Die Übereinstimmung der in verhaltens- und elektrophysiologischen Experimenten ermittelten Ergebnisse rechtfertigt es, auch bei den neuralen olfaktorischen Reaktionsschwellen von Riechschwellen zu sprechen.

References

Bretting, H. (1972): Die Bestimmung der Riechschwellen bei Igeln (*Erinaceus europaeus* L.) für einige Fettsäuren. Z. Säugetierkunde **37**, 286–311.

Gruch, W. (1957): Über die Riechfähigkeit bei Wanderratten. Zool. Jb., Abt. Physiol. **67**, 65–80.

Matthes, E. (1932): Geruchsdressuren an Meerschweinchen. Z. vergl. Physiol. **16**, 766–788.

Moulton, D. G. (1961): Studies in olfactory acuity. III. Relative detectability of n-aliphatic acetates by the rat. Quart. J. Exp. Psychol. **13**, 203–213.

Moulton, D. G.; Eayrs, J. T. (1966): Studies in olfactory acuity. II. Relative detectability of n-aliphatic alcohols by the rat. Quart. J. Exp. Psychol. **12**, 99–109.

Schmidt, C.; Schmidt, U. (1980): Changes of olfactory sensitivity during the estrus cycle in female laboratory mice. Chem. Senses **5**, 359–365.

— — (1982): Olfaction in pregnant and lactating mice. Z. Säugetierkunde **47**, 50–55.

Schmidt, U. (1978): Evoked-potential measurements of olfactory thresholds of laboratory mice *(Mus musculus)* to carboxylic acids. Chem. Senses Flav. **3**, 177–182.

Author's address: Dr. Christel Schmidt, Zoologisches Institut der Universität, Poppelsdorfer Schloß, D–530 Bonn

Habitat selection and fluctuations in numbers in a population of the arctic hare *(Lepus timidus)* on a subarctic fell in Finnish Forest Lapland

By E. Pulliainen

Receipt of Ms. 20. 11. 1981

Abstract

Habitat selection and population fluctuations were studied in the arctic hare *(Lepus timidus)* in the Värriötunturi fell area, East Finnish Forest Lapland, in the winters of 1968/69–1980/81. A transect survey indicated that the density of the population fluctuated in cycles of at least four years. Only the preferred habitats (i.e. lowland conifer-dominated mixed forests with birch and/or characterized by juniper) were inhabited during a population low, while in peak winters hares also occurred in less favoured habitats (e.g. mountain birch forests on windy slopes). The population decline is not triggered by predation. The movement activity of the hares when searching for food may increase considerably in the late winter at times of high populations.

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